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(54) Echinocandin binding domain of 1,3-Beta-glucan synthase

(57) The invention relates to a substantially purified ECB binding domain of 1,3- β -glucan synthase, comprising an at least 46 amino acid peptide fragment or fusion

protein of glucan synthase that binds echinocandins, useful in a method for identifying new antifungal compounds. Also disclosed are nucleic acid molecules that encode said peptide.

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Description

[0001] This invention claims the benefit of U.S. Provisional Application No. 60/068,658, filed December 23, 1997.

5 [0002] This invention relates to recombinant DNA technology. In particular the invention pertains to a fungal glucan synthase, and to a sub-region thereof that mediates echinocandin binding and antifungal activity. Also contemplated is the use of said echinocandin binding region in screens for compounds that bind glucan synthase.

10 [0003] The incidence of life-threatening fungal infections is increasing at an alarming rate. About 90% of nosocomial fungal infections are caused by species of *Candida*, with the remaining 10% being attributable to *Aspergillus*, *Cryptococcus*, and *Pneumocystis*. While effective antifungal compounds have been developed for *Candida*, there is growing concern over escalating resistance in other pathogenic fungi. Since anti-*Candida* compounds rarely are clinically effective against other fungi, new compounds are needed for effective antifungal therapy.

15 [0004] The present invention provides an echinocandin binding domain of a fungal 1,3,β-glucan synthase (hereinafter "glucan synthase") that is useful in identifying compounds that bind and inhibit glucan synthase activity. The compositions of this invention enable identification of new and better antifungal compounds.

[0005] In one embodiment the present invention relates to a nucleic acid molecule that encodes an echinocandin binding domain of fungal glucan synthase.

[0006] In another embodiment the present invention relates to a peptide that comprises an echinocandin binding site of fungal glucan synthase.

[0007] In another embodiment, the present invention relates to a method for identifying compounds that bind an echinocandin binding domain of fungal glucan synthase.

20 [0008] "ECB binding domain" or "ECB binding site" or "ECB binding fragment" refers to a subregion of the yeast glucan synthase molecule (i.e. product of *FKS1* gene in *S. cerevisiae*), wherein said subregion retains, either alone or in combination with another protein, for example, as a fusion protein, the capacity to bind echinocandins such as ECB. For example, in one embodiment the present invention relates to a subregion of SEQ ID NO:2 comprising amino acid residues 583 to 672. ECB binding fragments may be verified by any suitable test for binding to ECB or other echinocandin, or papulocandin, or related compounds.

25 [0009] The term "fusion protein" denotes a hybrid protein molecule not found in nature comprising a translational fusion or enzymatic fusion in which two or more different proteins or fragments thereof are covalently linked on a single polypeptide chain.

30 [0010] The term "plasmid" refers to an extrachromosomal genetic element. The starting plasmids herein are either commercially available, publicly available on an unrestricted basis, or can be constructed from available plasmids in accordance with published procedures. In addition, equivalent plasmids to those described are known in the art and will be apparent to the ordinarily skilled artisan.

35 [0011] "Recombinant DNA cloning vector" as used herein refers to any autonomously replicating agent, including, but not limited to, plasmids and phages, comprising a DNA molecule to which one or more additional DNA segments can or have been added.

[0012] The term "recombinant DNA expression vector" as used herein refers to any recombinant DNA cloning vector, for example a plasmid or phage, in which a promoter and other regulatory elements are present to enable transcription of the inserted DNA.

40 [0013] The term "vector" as used herein refers to a nucleic acid compound used for introducing exogenous DNA into host cells. A vector comprises a nucleotide sequence which may encode one or more protein molecules. Plasmids, cosmids, viruses, and bacteriophages, in the natural state or which have undergone recombinant engineering, are examples of commonly used vectors.

45 [0014] The terms "complementary" or "complementarity" as used herein refers to the capacity of purine and pyrimidine nucleotides to associate through hydrogen bonding in double stranded nucleic acid molecules. The following base pairs are complementary: guanine and cytosine; adenine and thymine; and adenine and uracil.

[0015] "Isolated nucleic acid compound" refers to any RNA or DNA sequence, however constructed or synthesized, which is locationally distinct from its natural location.

50 [0016] A "primer" is a nucleic acid fragment which functions as an initiating substrate for enzymatic or synthetic elongation of, for example, a nucleic acid molecule.

[0017] The term "promoter" refers to a DNA sequence which directs transcription of DNA to RNA.

[0018] A "probe" as used herein is a labeled nucleic acid compound which hybridizes with another nucleic acid compound.

55 [0019] The term "hybridization" as used herein refers to a process in which a single-stranded nucleic acid molecule joins with a complementary strand through nucleotide base pairing. "Selective hybridization" refers to hybridization under conditions of high stringency. The degree of hybridization depends upon, for example, the degree of complementarity, the stringency of hybridization, and the length of hybridizing strands.

[0020] The term "stringency" refers to hybridization conditions. High stringency conditions disfavor non-homologous

basepairing. Low stringency conditions have the opposite effect. Stringency may be altered, for example, by temperature and salt concentration.

[0021] "Low stringency" conditions comprise, for example, a temperature of about 37° C or less, a formamide concentration of less than about 50%, and a moderate to low salt (SSC) concentration; or, alternatively, a temperature of about 50° C or less, and a moderate to high salt (SSPE) concentration, for example 1M NaCl.

[0022] "High stringency" conditions comprise, for example, a temperature of about 42° C or less, a formamide concentration of less than about 20%, and a low salt (SSC) concentration; or, alternatively, a temperature of about 65° C, or less, and a low salt (SSPE) concentration. For example, high stringency conditions comprise hybridization in 0.5 M NaHPO₄, 7% sodium dodecyl sulfate (SDS), 1 mM EDTA at 65°C (Ausubel, F.M. et al. Current Protocols in Molecular Biology, Vol. I, 1989; Green Inc. New York, at 2.10.3).

[0023] "SSC" comprises a hybridization and wash solution. A stock 20X SSC solution contains 3M sodium chloride, 0.3M sodium citrate, pH 7.0.

[0024] "SSPE" comprises a hybridization and wash solution. A 1X SSPE solution contains 180 mM NaCl, 9mM Na₂HPO₄, 0.9 mM NaH₂PO₄ and 1 mM EDTA, pH 7.4.

[0025] "Substantially pure" used in reference to a peptide or protein means that said peptide or protein is separated from a large fraction of all other cellular and non-cellular molecules, including other protein molecules. A substantially pure preparation would be about at least 85% pure; preferably about at least 95% pure. For example, a "substantially pure" protein as described herein could be prepared by the IMAC protein purification method, or any other suitable method.

[0026] Cell walls are essential to the viability of fungi, but have no existence in mammalian cells. This makes synthesis of the fungal cell wall a useful target for antifungal compounds. Two polysaccharide polymers, chitin and 1,3-β-glucan, are essential components of fungal cell walls. Therefore, antibiotics that interfere with the synthesis of these polymers are useful in mycosis therapy. Polysaccharides have been estimated to account for as much as 80% to 90% of the *Saccharomyces cerevisiae* cell wall. The major cell wall polymers are glucan and mannan, and small amounts of chitin.

[0027] In *S. cerevisiae*, cell wall synthesis is thought to involve at least a subunit of glucan synthase, which is encoded by the *FKS1* gene (Douglas et al. *Proc. Nat. Acad. Sci.* 91, 12907-911, 1994). *FKS1* encodes a 215 kD integral membrane protein of 1876 amino acid residues that is the likely target of ECB and other echinocandins (*Id.*) For example; resistance to ECB and other echinocandins maps to the *FKS1* locus. More specifically, a domain of *FKS1*, which resides at amino acid residues 583 to 672 defines a cytoplasmic loop thought to be necessary and sufficient to comprise an echinocandin binding domain.

Gene Isolation Procedures

[0028] Those skilled in the art will recognize that the nucleic acids of this invention may be obtained by a plurality of applicable genetic and recombinant DNA techniques including, for example, polymerase chain reaction (PCR) amplification, or *de novo* DNA synthesis. (See e.g., J. Sambrook et al. Molecular Cloning, 2d Ed. Chap. 14 (1989)).

[0029] Skilled artisans will recognize that a nucleic acid encoding the ECB binding domain could be isolated by PCR amplification of any suitable genomic DNA or cDNA using oligonucleotide primers targeted to the appropriate region of *FKS1* (viz. encoding amino acid residues 587 to 672 of SEQ ID NO:2). The preferred template source for PCR amplification is *S. cerevisiae* genomic DNA. Methods for PCR amplification are widely known in the art. See e.g. PCR Protocols: A Guide to Method and Application, Ed. M. Innis et al., Academic Press (1990). The amplification reaction comprises genomic DNA, suitable enzymes, primers, and buffers, and is conveniently carried out in a DNA Thermal Cycler (Perkin Elmer Cetus, Norwalk, CT). A positive result is determined by detecting an appropriately-sized DNA fragment following agarose gel electrophoresis.

Protein Production Methods

[0030] The present invention also relates to a substantially purified peptide, or fusion protein, comprising a sub-region of glucan synthase that functions as an echinocandin binding site.

[0031] Skilled artisans will recognize that the proteins and peptides of the present invention can be synthesized by any number of different methods including solid phase chemical synthesis or recombinant methods. Both methods are described in U.S. Patent 4,617,149, incorporated herein by reference.

[0032] The principles of solid phase chemical synthesis are well known in the art and may be found in general texts in the area. See, e.g., H. Dugas and C. Penney, Bioorganic Chemistry (1981) Springer-Verlag, New York, 54-92. For example, peptides may be synthesized by solid-phase methodology utilizing an Applied Biosystems 430A peptide synthesizer (Applied Biosystems, Foster City, CA) and synthesis cycles supplied by Applied Biosystems. Protected amino acids, such as t-butoxycarbonyl-protected amino acids, and other reagents are commercially available from many chemical supply houses.

[0033] The peptide of the present invention can also be produced by recombinant DNA methods using a cloned nucleic acid. Recombinant methods are preferred if a high yield of the peptide is desired. Expression of a cloned nucleic acid can be carried out in a variety of suitable hosts, well known to those skilled artisan. For example, the cloned DNA is introduced into a host cell by any suitable means, well known to those skilled in the art. While chromosomal integration of the cloned nucleic acid is within the scope of the present invention, it is preferred that it comprise part of a suitable extra-chromosomally maintained expression vector.

5 [0034] The basic steps in the recombinant production of the peptides of this invention are:

- 10 a) constructing a natural, synthetic or semisynthetic DNA encoding said protein, peptide, or fusion protein;
- b) integrating said DNA into an expression vector in a manner suitable for expressing the protein, either alone or as a fusion protein;
- 15 c) transforming or otherwise introducing said vector into an appropriate eucaryotic or prokaryotic host cell, forming a recombinant host cell,
- d) culturing said recombinant host cell in a manner to express the protein; and
- e) recovering and substantially purifying the protein by any suitable means.

20 Expressing a Recombinant ECB Binding Domain in Prokaryotic and Eucaryotic Host Cells

[0035] In general, procaryotes are used for cloning DNA sequences and for constructing the vectors of the present invention. Procaryotes may also be used in the production of the ECB binding peptide. For example, the *Escherichia coli* K12 strain 294 (ATCC No. 31446) is particularly useful for the prokaryotic expression of foreign proteins. Other strains of *E. coli*, bacilli such as *Bacillus subtilis*, enterobacteriaceae such as *Salmonella typhimurium* or *Serratia marcescens*, various Pseudomonas species and other bacteria, such as *Streptomyces*, may also be employed as host cells in the cloning and expression of the recombinant proteins of this invention.

[0036] Promoter sequences suitable for driving the expression of genes in procaryotes include β -lactamase [e.g. 30 vector pGX2907, ATCC 39344, contains a replicon and β -lactamase gene], lactose systems [Chang et al., *Nature* (London), 275:615 (1978); Goeddel et al., *Nature* (London), 281:544 (1979)], alkaline phosphatase, and the tryptophan (trp) promoter system [vector pATH1 (ATCC 37695) which is designed to facilitate expression of an open reading frame as a trpE fusion protein under the control of the trp promoter]. Hybrid promoters such as the tac promoter (isolatable from plasmid pDR540, ATCC-37282) are also suitable. Still other bacterial promoters, whose nucleotide sequences 35 are generally known, enable one of skill in the art to ligate such promoter sequences to DNA encoding the proteins of the instant invention using linkers or adapters to supply any required restriction sites. Promoters for use in bacterial systems also will contain a Shine-Dalgarno sequence operably-linked to the DNA encoding the desired polypeptides. These examples are illustrative rather than limiting.

[0037] The peptides of this invention may be synthesized de novo, or they may be produced as a fusion protein 40 comprising the peptide of interest (viz. ECB binding fragment) as a translational fusion with another protein or peptide that may be removable by enzymatic or chemical cleavage. It is often observed that expression as a fusion protein prolongs the lifespan, increases the yield of a desired peptide, and provides a convenient means of purifying the protein. A variety of peptidases (e.g. enterokinase and thrombin) which cleave a polypeptide at specific sites or digest the peptides from the amino or carboxy termini (e.g. diaminopeptidase) of the peptide chain are known. Furthermore, 45 particular chemicals (e.g. cyanogen bromide) cleave a polypeptide chain at specific sites. The skilled artisan will appreciate the modifications necessary to the amino acid sequence (and synthetic or semisynthetic coding sequence if recombinant means are employed) to incorporate site-specific internal cleavage sites. See e.g., P. Carter, "Site Specific Proteolysis of Fusion Proteins", Chapter 13, in *Protein Purification: From Molecular Mechanisms to Large Scale Processes*, American Chemical Society, Washington, D.C. (1990).

[0038] The present invention contemplates ECB binding fusion proteins comprising a fragment of glucan synthase 50 in fusion with another protein, thereby facilitating isolation, purification, and assay of said ECB binding fragment. A variety of embodiments and methods for producing fusion proteins are known in the art and are suitable for the present invention. For example, foreign proteins may be fused with the carboxy terminus of Sj26, a 26 kDa glutathione S-transferase (GST), encoded by the parasitic helminth *Schistosoma japonicum*. Such fusion proteins may be expressed 55 in *E. coli* or other suitable procaryote, or in eucaryotic hosts, such as yeast. In this regard, the method and vectors of Smith and Johnson are especially suitable (*Gene*, 67, 31-40, 1988), the entire contents of which is incorporated by reference. It is desirable that the fusion protein remain in solution to facilitate ease of purification.

[0039] In addition to procaryotes, a variety of mammalian cell systems and eucaryotic microorganisms such as yeast

are suitable host cells for the recombinant expression of proteins or fusion proteins. The yeast *Saccharomyces cerevisiae* is the most commonly used eucaryotic microorganism. A number of other yeasts such as *Kluyveromyces lactis* and *Schizosaccharomyces pombe* are also suitable. For expression in *Saccharomyces*, the plasmid YRp7 (ATCC-40053), for example, may be used. See, e.g., D. Stinchcomb, et al., *Nature*, 282:39 (1979); J. Kingsman, et al., *Gene*, 7:141 (1979); S. Tschemper, et al., *Gene*, 10:157 (1980). Plasmid YRp7 contains the TRP1 gene which provides a selectable marker for use in a trp auxotrophic mutant. For expression in *S. pombe* suitable vectors include those containing the *nmt1* promoter as well as the *adh* promoter and the SV40 promoter (See e.g. S. Forsburg, *Nuc. Acid. Res.* 21, 2955, 1993).

10 Purification of Recombinantly-Produced ECB Binding Peptide

[0040] An expression vector comprising a cloned nucleic acid encoding an ECB binding domain is transformed or transfected into a suitable host cell using standard methods. Cells that contain the vector are propagated under conditions suitable for expression of the peptide. If the gene is controlled by an inducible promoter, suitable growth conditions should incorporate the appropriate inducer. Recombinantly-produced peptide may be purified from cellular extracts of transformed cells by any suitable means. In one process for peptide purification, the gene is modified at the 5' end to incorporate several histidine residues at the amino terminus of the peptide. This "histidine tag" enables a single-step protein purification method referred to as "immobilized metal ion affinity chromatography" (IMAC), essentially as described in U.S. Patent 4,569,794 which hereby is incorporated by reference. The IMAC method enables rapid isolation of substantially pure peptide starting from a crude cellular extract.

[0041] Other embodiments of the present invention comprise isolated nucleic acid sequences that comprise SEQ ID NO:2, wherein said sequences encode amino acid residues 583 to 672 of SEQ ID NO:2. As skilled artisans will recognize, the amino acid compounds of the invention can be encoded by a multitude of different nucleic acid sequences because most of the amino acids are encoded by more than one codon due to the degeneracy of the genetic code. Because these alternative nucleic acid sequences would encode the same amino acid sequences, the present invention further comprises these alternate nucleic acid sequences.

[0042] Nucleic acids encoding an ECB binding domain of SEQ ID NO:2 may be produced by synthetic methods. Fragments of the proteins disclosed herein may be generated by any number of suitable techniques, including chemical synthesis of a suitable portion of SEQ ID NO:2, proteolytic digestion of SEQ ID NO:2, or most preferably, by recombinant DNA mutagenesis techniques, well known to the skilled artisan. See, e.g. K. Struhl, "Reverse biochemistry: Methods and applications for synthesizing yeast proteins *in vitro*," *Meth. Enzymol.* 194, 520-535. For example, in a preferred method, a nested set of deletion mutations are introduced into the intact *FKS1* gene (SEQ ID NO:1) encoding the native glucan synthase protein, such that varying amounts of the protein coding region are deleted, either from the amino terminal end, or from the carboxyl end of the protein molecule, and wherein said deletions produce molecules that retain amino acid residues from about 605 to 650, or more preferably amino acid residues from about 583 to 672 of SEQ ID NO:2. Internal fragments of the intact protein can also be produced in which both the carboxyl and amino terminal ends are removed. Several nucleases can be used to generate deletions, for example *Bal* 31, or in the case of a single stranded nucleic acid molecule, mung bean nuclease. For simplicity, it is preferred that the intact *FKS1* gene be cloned into a single-stranded cloning vector, such as bacteriophage M13, or equivalent. If desired, the resulting gene deletion fragments can be subcloned into any suitable vector for propagation and expression of said fragments in any suitable host cell. It is preferred that the fragments be subcloned into a plasmid, for example pGEX-1 (Smith & Johnson, *Gene*, 67, 31, 1988), enabling the production of a fusion protein comprising an ECB binding domain.

[0043] The present invention provides fragments of the intact glucan synthase protein disclosed herein wherein said fragments retain the ability to bind ECB or other echinocandin or papulocandin.

[0044] ECB binding fragments of the intact proteins disclosed herein may be produced as described above, preferably using cloning techniques to produce fragments of the intact *FKS1* gene. Peptide fragments of glucan synthase or fusion proteins comprising a peptide fragment of glucan synthase may be tested for binding activity using any suitable assay.

[0045] The synthesis of nucleic acids is well known in the art. See, e.g., E.L. Brown, R. Belagaje, M.J. Ryan, and H.G. Khorana, *Methods in Enzymology*, 68:109-151 (1979). The nucleic acids of this invention could be generated using a conventional DNA synthesizing apparatus, such as the Applied Biosystems Model 380A or 380B DNA synthesizers (Applied Biosystems, Inc., 850 Lincoln Center Drive, Foster City, CA 94404) which employ phosphoramidite chemistry. Alternatively, phosphotriester chemistry may be employed to synthesize the nucleic acids of this invention. [See, e.g., M.J. Gait, ed., *Oligonucleotide Synthesis, A Practical Approach*, (1984).]

[0046] In an alternative methodology, namely PCR, the nucleic acids comprising a portion or all of SEQ ID NO:1 can be generated from *S. cerevisiae* genomic DNA using suitable oligonucleotide primers complementary to SEQ ID NO: 1 or region therein, as described in U.S. Patent No. 4,889,818, which hereby is incorporated by reference. Suitable protocols for performing the PCR are disclosed in, for example, *PCR Protocols: A Guide to Method and Applications*, Ed. Michael A. Innis et al., Academic Press, Inc. (1990).

- [0047] The ribonucleic acids of the present invention may be prepared using the polynucleotide synthetic methods discussed *supra*, or they may be prepared enzymatically using RNA polymerase to transcribe a DNA template.
- [0048] The most preferred systems for preparing the ribonucleic acids of the present invention employ the RNA polymerase from the bacteriophage T7 or the bacteriophage SP6. These RNA polymerases are highly specific, requiring the insertion of bacteriophage-specific sequences at the 5' end of the template to be transcribed. See, J. Sambrook, *et al., supra*, at 18.82-18.84.
- [0049] This invention also provides nucleic acids, RNA or DNA, which are complementary to the nucleic acids encoding the ECB binding domain of SEQ ID NO:2.
- [0050] The present invention also provides probes and primers useful for a variety of molecular biology techniques including, for example, hybridization screens of genomic or subgenomic libraries. A nucleic acid compound comprising SEQ ID NO:1, or a complementary sequence thereof, or a fragment thereof, and which is at least 18 base pairs in length, and which will selectively hybridize to *Saccharomyces cerevisiae* DNA or mRNA encoding FKS1, is provided. Preferably, the 18 or more base pair compound is DNA. A probe or primer length of at least 18 base pairs is dictated by theoretical and practical considerations. See e.g. B. Wallace and G. Miyada, "Oligonucleotide Probes for the Screening of Recombinant DNA Libraries," In Methods in Enzymology, Vol. 152, 432-442, Academic Press (1987).
- [0051] These probes and primers can be prepared by enzymatic methods well known to those skilled in the art (See e.g. Sambrook *et al. supra*). In a most preferred embodiment these probes and primers are synthesized using chemical means as described above.
- [0052] Another aspect of the present invention relates to recombinant DNA cloning vectors and expression vectors comprising the nucleic acids of the present invention. Many of the vectors encompassed within this invention are described above. The preferred nucleic acid vectors are those which comprise DNA. The most preferred recombinant DNA vectors comprise nucleic acid encoding the ECB binding domain of SEQ ID NO:2.
- [0053] The skilled artisan understands that choosing the most appropriate cloning vector or expression vector depends upon a number of factors including the availability of restriction enzyme sites, the type of host cell into which the vector is to be transfected or transformed, the purpose of the transfection or transformation (e.g., stable transformation as an extrachromosomal element, or integration into the host chromosome), the presence or absence of readily assayable or selectable markers (e.g., antibiotic resistance and metabolic markers of one type and another), and the number of copies of the gene to be present in the host cell.
- [0054] Vectors suitable to carry the nucleic acids of the present invention comprise RNA viruses, DNA viruses, lytic bacteriophages, lysogenic bacteriophages, stable bacteriophages, plasmids, viroids, and the like. The most preferred vectors are plasmids.
- [0055] When preparing an expression vector the skilled artisan understands that there are many variables to be considered, for example, whether to use a constitutive or inducible promoter. Inducible promoters are preferred because they enable high level, regulatable expression of an operably linked gene. The skilled artisan will recognize a number of inducible promoters which respond to a variety of inducers, for example, carbon source, metal ions, heat, and others. The practitioner also understands that the amount of nucleic acid or protein to be produced dictates, in part, the selection of the expression system. The addition of certain nucleotide sequences is useful for directing the localization of a recombinant protein. For example, a sequence encoding a signal peptide preceding the coding region of a gene, is useful for directing the extra-cellular export of a resulting polypeptide.
- [0056] The present invention also provides a method for constructing a recombinant host cell capable of expressing the ECB binding domain of SEQ ID NO:2, said method comprising transforming or otherwise introducing into a host cell a recombinant DNA vector that comprises an isolated DNA sequence encoding amino acid residues from about 583 to 672 of SEQ ID NO:2. Suitable host cells include any strain of *E. coli* or *S. cerevisiae* that can accommodate high level expression of an exogenously introduced gene. Transformed host cells may be cultured under conditions well known to skilled artisans such that the ECB binding domain is expressed, thereby producing ECB binding peptide in the recombinant host cell.
- [0057] Agents that bind the ECB binding domain may identify new antifungal compounds. Substances that bind the ECB binding peptide can be identified by contacting the peptide with a test compound and monitoring the interaction by any suitable means.
- [0058] The instant invention provides a screening method for discovering compounds that bind the ECB binding peptide, said method comprising the steps of:
- 55 a) preparing the binding peptide, preferably as a fusion protein;
 - b) exposing said peptide or protein to a test compound; and
 - c) quantifying the binding of said compound to said peptide by any suitable means.

[0059] In one embodiment, a protein comprising a fusion of the 89 amino acid residue ECB binding domain of SEQ ID NO:2 and a GST protein is expressed in yeast or *E. coli*, and purified for use in a microtiter plate ELISA screen. The ELISA screen enables an assay for the displacement of ECB from the ECB binding domain by a test compound. Bound ECB, or ECB free in solution can be detected using an ECB-specific antibody prepared using standard methods.

5 If a test compound displaces ECB from the binding domain there will be a diminution in the ELISA signal. This method involves coating the wells of a microtiter plate with, for example, a GST-FKS1 fusion protein. After blocking residual binding sites the plates are rinsed to remove unbound fusion protein and then incubated with ECB. After rinsing again to remove unbound ECB, a test compound is added, incubated, and rinsed to remove unbound test compound or displaced ECB. The plates are then incubated with an antibody against ECB that is covalently linked to alkaline phosphatase (anti-ECB-AP). The plates are developed by adding an appropriate substrate, e.g. p-nitrophenyl phosphate for colorimetric detection, or 4-methylumbelliferyl phosphate for fluorimetric detection.

10 [0060] This screening method may be adapted to automated procedures such as a PANDEX® (Baxter-Dade Diagnostics) system, allowing for efficient high-volume screening of potential therapeutic agents.

15 [0061] In such a screening protocol an ECB binding peptide is prepared as described herein, preferably using recombinant DNA technology. A test compound is introduced into the reaction vessel containing the peptide.

20 [0062] Skilled artisans will recognize that IC₅₀ values are dependent on the selectivity of the compound tested. For example, a compound with an IC₅₀ which is less than 10 nM is generally considered an excellent candidate for drug therapy. However, a compound which has a lower affinity, but is selective for a particular target, may be an even better candidate. The skilled artisan will recognize that any information regarding inhibitory activity or selectivity of a particular compound is beneficial in the pharmaceutical arts.

25 [0063] The following examples more fully describe the present invention. Those skilled in the art will recognize that the particular reagents, equipment, and procedures described are merely illustrative and are not intended to limit the present invention in any manner.

EXAMPLE 1

Expression Vector Encoding the ECB Binding Domain

30 [0064] A vector for expressing a fusion protein in yeast comprising the ECB binding domain of yeast glucan synthase and glutathione S-transferase (GST) is prepared as follows. Plasmid pGEX-1 (Smith and Johnson, Gene, 67, 31-40, 1988) is an *E. coli* expression vector that comprises the *tac* promoter and the complete coding sequence of Sj26 (viz. GST), in which the normal termination codon is replaced by a polylinker containing unique BamH1, Sma1, and EcoR1 restriction sites, followed by a termination codon in all 3 reading frames. A fragment of pGEX-1 containing the described GST gene is isolated by any suitable subcloning method, well known to the skilled artisan. It is convenient, but not necessary, for subsequent cloning steps, to attach to the fragment containing the GST gene of pGEX-1 oligonucleotides containing specific restriction enzyme sites. For convenience, the GST fragment thus described is cloned into the multiple cloning site of yeast expression vector pREP1 (K. Maundrell, *J. Biol. Chem.* 265, 10857, 1990), in the correct orientation, downstream of the LEU2 gene, and *nmt1* promoter. pREP1 also contains an ARS element for replication in the host yeast. The resulting plasmid, pREP1-GST, is linearized at any one or more of BarnH1, Sma1, or EcoR1 sites at the 3' end of the GST fragment, for cloning in the ECB binding domain.

35 [0065] A DNA fragment encoding the ECB binding domain of SEQ ID NO:2 is conveniently prepared by PCR. Oligonucleotide primers are prepared for priming DNA synthesis on opposite strands from nucleotide positions 1747 through 2016 of SEQ ID NO:1. It is convenient to include suitable restriction sites at the appropriate 5' or 3' end of the PCR primers for subsequent cloning. The ECB binding fragment so prepared is purified by any suitable method, for example, isolation by gel electrophoresis. The purified ECB binding fragment is ligated into pREP1-GST so that the ECB binding fragment is linked to the 3' end of the GST gene. This construct, pREP1-GST-ECB, produces a fusion protein comprising a GST-ECB binding domain.

EXAMPLE 2

E. coli Expression Vector Encoding the ECB Binding Domain

50 [0066] A vector for expressing a fusion protein in *E. coli* comprising the ECB binding domain of yeast glucan synthase and glutathione S-transferase (GST) is prepared as follows. Plasmid pGEX-1 (Smith and Johnson, Gene, 67, 31-40, 1988) is an *E. coli* expression vector that comprises the *tac* promoter and the complete coding sequence of Sj26 (viz. GST), in which the normal termination codon is replaced by a polylinker containing unique BarnH1, Sma1, and EcoR1 restriction sites, followed by a termination codon in all 3 reading frames.

55 [0067] A DNA fragment encoding the ECB binding domain of SEQ ID NO:2 is conveniently prepared by PCR. Oli-

gonucleotide primers are prepared for priming DNA synthesis on opposite strands, from nucleotide positions 1747 through 2016 of SEQ ID NO:1. It is convenient to design into the oligonucleotide sequence suitable restriction sites at the termini for subsequent cloning steps. The ECB binding fragment so prepared is purified by any suitable method, for example, isolation from a gel following electrophoresis. The purified ECB binding fragment is ligated into pGEX-1
 5 so that the ECB binding fragment is linked to the 3' end of the GST gene. This construct, pGST-ECB, produces a fusion protein comprising a GST-ECB binding domain.

EXAMPLE 3

10 Expression of ECB Fusion Protein in *S. pombe*

[0068] Expression plasmid pREP1-GST-ECB (Example 1) is transformed into any suitable strain of *S. pombe*, for example, a leul strain (See e.g. R. Sikorski & P. Hieter, *Genetics*, 122, 19-26, 1989; K. Maundrell, *J. Biol. Chem.* 265, 10857, 1990) using standard methods, for example, spheroplast transformation, or lithium acetate transformation (See
 15 e.g. Sambrook *et al. Supra*; Okazaki *et al. Nuc. Acid Res.* 18, 6485-89 (1990); Moreno *et al. Meth. Enzym.* 194, 795-823 (1991). Transformants, chosen at random, are tested for the presence of the plasmid by agarose gel electrophoresis using quick plasmid preparations. *Id.* Transformants are grown overnight under conditions suitable to induce the *nmt1*
 20 promoter, for example, in minimal medium lacking thiamine (Beach & Nurse, *Nature*, 290, 140, 1981). The overnight culture was diluted into fresh medium and allowed to grow to mid-log phase. The induced-culture was pelleted by centrifugation in preparation for protein purification.

EXAMPLE 4

Affinity Purification of a Recombinantly-Produced ECB Binding Domain

[0069] Overnight cultures of transformed *E. coli* or yeast cells, (See e.g. Example 3), are lysed by sonication with glass beads, or by spheroplast formation in MTPBS (150 mM NaCl, 16 mM Na₂HPO₄, 4 mM NaH₂PO₄ (pH 7.3) and including 1% Triton X-100 (BDH Chemicals). Lysed cells are subjected to centrifugation at 10,000 x g for 5 minutes at 4° C. The supernatant is mixed on a rotating platform with 1 to 2 ml 50% glutathione-agarose beads (sulphur linkage,
 30 Sigma). After absorption for 2 minutes, beads are collected by brief centrifugation at 500 x g and washed 3 times with 50 ml MTPBS. Fusion protein is eluted by competition with free glutathione, using 2 x 2 minute washes with 1 bead volume of 50 mM Tris HCl, pH 8, containing 5 mM reduced glutathione (Sigma), pH 7.5.

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Annex to the description

[0070]

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SEQUENCE LISTING

(1) GENERAL INFORMATION:

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- (i) APPLICANT: ELI LILLY AND COMPANY
- (B) STREET: Lilly Corporate Center
- (C) CITY: Indianapolis
- (D) STATE: Indiana
- (E) COUNTRY: United States of America
- (F) ZIP: 46285

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- (ii) TITLE OF INVENTION: Echinocandin Binding Site of 1,3-B-Glucan Synthase

- (iii) NUMBER OF SEQUENCES: 2

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- (iv) CORRESPONDENCE ADDRESS:
 - (A) ADDRESSEE: A. M. Denholm
 - (B) STREET: Erl Wood Manor
 - (C) CITY: Windlesham
 - (D) STATE: Surrey
 - (E) COUNTRY: United Kingdom
 - (F) ZIP: GU20 6PH

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- (v) COMPUTER READABLE FORM:
 - (A) MEDIUM TYPE: Floppy disk
 - (B) COMPUTER: IBM PC compatible
 - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
 - (D) SOFTWARE: PatentIn Release #1.0, Version #1.30

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(2) INFORMATION FOR SEQ ID NO:1:

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- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 5631 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: DNA (genomic)

- (iii) HYPOTHETICAL: NO

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- (iv) ANTI-SENSE: NO

- (ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1..5628

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- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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| ATG AAC ACT GAT CAA CAA CCT TAT CAG GGC CAA ACG GAC TAT ACC CAG Met Asn Thr Asp Gln Gln Pro Tyr Gln Gly Gln Thr Asp Tyr Thr Gln | 48 |
| 1 5 10 15 | |

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| GGA CCA GGT AAC GGG CAA AGT CAG GAA CAA GAC TAT GAC CAA TAT GGC Gly Pro Gly Asn Gly Gln Ser Gln Glu Gln Asp Tyr Asp Gln Tyr Gly | 96 |
| 20 25 30 | |

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| CAG CCT TTG TAT CCT TCA CAA GCT GAT GGT TAC TAC GAT CCA AAT GTC Gln Pro Leu Tyr Pro Ser Gln Ala Asp Gly Tyr Tyr Asp Pro Asn Val | 144 |
| 35 40 45 | |

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|----|---|-----|
| | GCT GCT GGT ACT GAA GCT GAT ATG TAT GGT CAA CAA CCA CCA AAC GAG Ala Ala Gly Thr Glu Ala Asp Met Tyr Gly Gln Gln Pro Pro Asn Glu 50 55 60 | 192 |
| 5 | TCT TAC GAC CAA GAC TAC ACA AAC GGT GAA TAC TAT GGT CAA CCG CCA Ser Tyr Asp Gln Asp Tyr Thr Asn Gly Glu Tyr Tyr Gly Gln Pro Pro 65 70 75 80 | 240 |
| 10 | AAT ATG GCT GCT CAA GAC GGT GAA AAC TTC TCG GAT TTT AGC AGT TAC Asn Met Ala Ala Gln Asp Gly Glu Asn Phe Ser Asp Phe Ser Ser Tyr 85 90 95 | 288 |
| | GGC CCT CCT GGA ACA CCT GGA TAT GAT AGC TAT GGT GGT CAG TAT ACC Gly Pro Pro Gly Thr Pro Gly Tyr Asp Ser Tyr Gly Gly Gln Tyr Thr 100 105 110 | 336 |
| 15 | GCT TCT CAA ATG AGT TAT GGA GAA CCA AAT TCG TCG GGT ACC TCG ACT Ala Ser Gln Met Ser Tyr Gly Glu Pro Asn Ser Ser Gly Thr Ser Thr 115 120 125 | 384 |
| | CCA ATT TAC GGT AAT TAT GAC CCA AAT GCT ATC GCT ATG GCT TTG CCA Pro Ile Tyr Gly Asn Tyr Asp Pro Asn Ala Ile Ala Met Ala Leu Pro 130 135 140 | 432 |
| 20 | AAT GAA CCT TAT CCC GCT TGG ACT GCT GAC TCT CAA TCT CCC GTT TCG Asn Glu Pro Tyr Pro Ala Trp Thr Ala Asp Ser Gln Ser Pro Val Ser 145 150 155 160 | 480 |
| 25 | ATC GAG CAA ATC GAA GAT ATC TTT ATT GAT TTG ACC AAC AGA CTC CGG Ile Glu Gln Ile Glu Asp Ile Phe Ile Asp Leu Thr Asn Arg Leu Gly 165 170 175 | 528 |
| | TTC CAA AGA GAC TCC ATG AGA AAT ATG TTT GAT CAT TTT ATG GTT CTC Phe Gln Arg Asp Ser Met Arg Asn Met Phe Asp His Phe Met Val Leu 180 185 190 | 576 |
| 30 | TTG GAC TCT AGG TCC TCG AGA ATG TCT CCT GAT CAA GCT TTA CTA TCT Leu Asp Ser Arg Ser Arg Met Ser Pro Asp Gln Ala Leu Leu Ser 195 200 205 | 624 |
| | TTA CAT GCC GAC TAC ATT GGT GGC GAT ACT GCT AAC TAT AAA AAA TGG Leu His Ala Asp Tyr Ile Gly Gly Asp Thr Ala Asn Tyr Lys Lys Trp 210 215 220 | 672 |
| 35 | TAT TTT GCT GCT CAG TTA GAT ATG GAT GAT GAA ATT GGT TTT AGA AAT Tyr Phe Ala Ala Gln Leu Asp Met Asp Asp Glu Ile Gly Phe Arg Asn 225 230 235 240 | 720 |
| 40 | ATG AGT CTT GGA AAA CTC TCA AGG AAG GCA AGA AAA GCT AAG AAG AAA Met Ser Leu Gly Lys Leu Ser Arg Lys Ala Arg Lys Ala Lys Lys Lys 245 250 255 | 768 |
| | AAC AAG AAA GCA ATG GAA GAG GCC AAT CCC GAA GAC ACT GAA GAA ACT Asn Lys Lys Ala Met Glu Glu Ala Asn Pro Glu Asp Thr Glu Glu Thr 260 265 270 | 816 |
| 45 | TTA AAC AAA ATT GAA GGC GAC AAC TCC CTA GAG GCT GCT GAT TTT AGA Leu Asn Lys Ile Glu Gly Asp Asn Ser Leu Glu Ala Ala Asp Phe Arg 275 280 285 | 864 |
| | TGG AAG CCC AAG ATG AAC CAG TTG TCT CCC CTG GAA AGA GTT CGT CAT Trp Lys Ala Lys Met Asn Gln Leu Ser Pro Leu Glu Arg Val Arg His 290 295 300 | 912 |
| 50 | | |

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|----|---|------|
| | ATC GCC TTA TAT CTG TTA TGT TGG GGT GAA GCT AAT CAA GTC AGA TTC Ile Ala Leu Tyr Leu Leu Cys Trp Gly Glu Ala Asn Gln Val Arg Phe 305 310 315 320 | 960 |
| 5 | ACT GCT GAA TGT TTA TGT TTT AFC TAC AAG TGT GCT CTT GAC TAC TAC TTG Thr Ala Glu Cys Leu Cys Phe Ile Tyr Lys Cys Ala Leu Asp Tyr Leu 325 330 335 | 1008 |
| 10 | GAT TCC CCT CTT TGC CAA CAA CGC CAA GAA CCT ATG CCA GAA GGT GAT Asp Ser Pro Leu Cys Gln Gln Arg Gln Glu Pro Met Pro Glu Gly Asp 340 345 350 | 1056 |
| | TTC TTG AAT AGA GTC ATT ACG CCA ATT TAT CAT TTC ATC AGA AAT CAA Phe Leu Asn Arg Val Ile Thr Pro Ile Tyr His Phe Ile Arg Asn Gln 355 360 365 | 1104 |
| 15 | GTT TAT GAA ATT GTT GAT GGT CGT TTT GTC AAG CGT GAA AGA GAT CAT Val Tyr Glu Ile Val Asp Gly Arg Phe Val Lys Arg Glu Arg Asp His 370 375 380 | 1152 |
| | AAC AAA ATT GTC GGT TAT GAT GAT TTA AAC CAA TTG TTC TGG TAT CCA Asn Lys Ile Val Gly Tyr Asp Asp Leu Asn Gln Leu Phe Trp Tyr Pro 385 390 395 400 | 1200 |
| 20 | GAA GGT ATT GCA AAG ATT GTT CTT GAA GAT GGA ACA AAA TTG ATA GAA Glu Gly Ile Ala Lys Ile Val Leu Glu Asp Gly Thr Lys Leu Ile Glu 405 410 415 | 1248 |
| 25 | CTC CCA TTG GAA GAA CGT TAT TTA AGA TTA GGC GAT GTC GTC TGG GAT Leu Pro Leu Glu Glu Arg Tyr Leu Arg Leu Gly Asp Val Val Trp Asp 420 425 430 | 1296 |
| | GAT GTA TTC TTC AAA ACA TAT AAA GAG ACC CGT ACT TGG TTA CAT TTG Asp Val Phe Phe Lys Thr Tyr Lys Glu Thr Arg Thr Trp Leu His Leu 435 440 445 | 1344 |
| 30 | GTC ACC AAC TTC AAC CGT ATT TGG GTT ATG CAT ATC TCC ATT TTT TGG Val Thr Asn Phe Asn Arg Ile Trp Val Met His Ile Ser Ile Phe Trp 450 455 460 | 1392 |
| | ATG TAC TTT GCA TAT AAT TCA CCA ACA TTT TAC ACT CAT AAC TAT CAA Met Tyr Phe Ala Tyr Asn Ser Pro Thr Phe Tyr Thr His Asn Tyr Gln 465 470 475 480 | 1440 |
| 35 | CAA TTG GTC GAC AAC CAA CCT TTG GCT GCT TAC AAG TGG GCA TCT TGC Gln Leu Val Asp Asn Gln Pro Leu Ala Ala Tyr Lys Trp Ala Ser Cys 485 490 495 | 1488 |
| 40 | GCA TTA GGT GGT ACT GTC GCA AGT TTG ATT CAA ATT GTC GCT ACT TTG Ala Leu Gly Gly Thr Val Ala Ser Leu Ile Gln Ile Val Ala Thr Leu 500 505 510 | 1536 |
| | TGT GAA TGG TCA TTC GTT CCA AGA AAA TGG GCT GGT GCT CAA CAT CTA Cys Glu Trp Ser Phe Val Pro Arg Lys Trp Ala Gly Ala Gln His Leu 515 520 525 | 1584 |
| 45 | TCT CGT AGA TTC TGG TTT TTA TGC ATC ATC TTT GGT ATT AAT TTG GGT Ser Arg Arg Phe Trp Phe Leu Cys Ile Ile Phe Gly Ile Asn Leu Gly 530 535 540 | 1632 |
| | CCT ATT ATT TTT GTT TTT GCT TAC GAC AAA GAT ACA GTC TAC TCC ACT Pro Ile Ile Phe Val Phe Ala Tyr Asp Lys Asp Thr Val Tyr Ser Thr 545 550 555 560 | 1680 |
| 50 | GCT GCA CAC GTT GTT GCT GCT GTT ATG TTC TTT GTT GCG GTT GCT ACC | 1728 |

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| | Ala Ala His Val Val Ala Ala Val Met Phe Phe Val Ala Val Ala Thr | |
| | 565 570 575 | |
| 5 | ATC ATA TTC TTC TCC ATT ATG CCA TTG GGG GGG TTG TTT ACG TCA TAT Ile Ile Phe Phe Ser Ile Met Pro Leu Gly Gly Leu Phe Thr Ser Tyr 580 585 590 | 1776 |
| | ATG AAA AAA TCT ACA AGG CGT TAT GTT GCA TCT CAA ACA TTC ACT GCT Met Lys Lys Ser Thr Arg Arg Tyr Val Ala Ser Gln Thr Phe Thr Ala 595 600 605 | 1824 |
| 10 | GCA TTT GCC CCT CTA CAT GGG TTA GAT AGA TGG ATG TCC TAT TTA GTT Ala Phe Ala Pro Leu His Gly Leu Asp Arg Trp Met Ser Tyr Leu Val 610 615 620 | 1872 |
| 15 | TGG GTT ACT GTT TTT GCT GCC AAA TAT TCA GAA TCG TAC TAC TTT TTA Trp Val Thr Val Phe Ala Ala Lys Tyr Ser Glu Ser Tyr Tyr Phe Leu 625 630 635 640 | 1920 |
| | GTT TTA TCT TTG AGA GAT CCA ATT AGA ATT TTG TCC ACC ACT GCA ATG Val Leu Ser Leu Arg Asp Pro Ile Arg Ile Leu Ser Thr Thr Ala Met 645 650 655 | 1968 |
| 20 | AGG TGT ACA GGT GAA TAC TGG TGG GGT GCG GTA CTT TGT AAA GTG CAA Arg Cys Thr Gly Glu Tyr Trp Trp Gly Ala Val Leu Cys Lys Val Gln 660 665 670 | 2016 |
| | CCC AAG ATT GTC TTA GGT TTG GTT ATC GCT ACC GAC TTC ATT CTT TTC Pro Lys Ile Val Leu Gly Leu Val Ile Ala Thr Asp Phe Ile Leu Phe 675 680 685 | 2064 |
| 25 | TTC TTG GAT ACC TAC TTA TGG TAC ATT ATT GTG AAT ACC ATT TTC TCT Phe Leu Asp Thr Tyr Leu Trp Tyr Ile Ile Val Asn Thr Ile Phe Ser 690 695 700 | 2112 |
| 30 | CTT GGG AAA TCT TTC TAT TTA GGT ATT TCT ATC TTA ACA CCA TGG AGA Val Gly Lys Ser Phe Tyr Leu Gly Ile Ser Ile Leu Thr Pro Trp Arg 705 710 715 720 | 2160 |
| | AAT ATC TTC ACA AGA TTG CCA AAA AGA ATA TAC TCC AAG ATT TTG GCT Asn Ile Phe Thr Arg Leu Pro Lys Arg Ile Tyr Ser Lys Ile Leu Ala 725 730 735 | 2208 |
| 35 | ACT ACT GAT ATG GAA ATT AAA TAC AAA CCA AAG GTT TTG ATT TCT CAA Thr Thr Asp Met Glu Ile Lys Tyr Lys Pro Lys Val Leu Ile Ser Gln 740 745 750 | 2256 |
| | GTA TGG AAT GCC ATC ATT ATT TCA ATG TAC AGA GAA CAT CTC TTA GCC Val Trp Asn Ala Ile Ile Ser Met Tyr Arg Glu His Leu Leu Ala 755 760 765 | 2304 |
| 40 | ATC GAC CAT GTA CAA AAA TTA CTA TAT CAT CAA GTT CCA TCT GAA ATC Ile Asp His Val Gln Lys Leu Leu Tyr His Gln Val Pro Ser Glu Ile 770 775 780 | 2352 |
| | GAA GGT AAA AGA ACT TTG AGA GCT CCT ACC TTC TTT GTT TCT CAA GAT Glu Gly Lys Arg Thr Leu Arg Ala Pro Thr Phe Phe Val Ser Gln Asp 785 790 795 800 | 2400 |
| | GAC AAT AAT TTT GAG ACT GAA TTT TTC CCT AGG GAT TCA GAG GCT GAG Asp Asn Asn Phe Glu Thr Glu Phe Phe Pro Arg Asp Ser Glu Ala Glu 805 810 815 | 2448 |
| 50 | CGT CGT ATT TCT TTC TTT GCT CAA TCT TTG TCT ACT CCA ATT CCC GAA Arg Arg Ile Ser Phe Phe Ala Gln Ser Leu Ser Thr Pro Ile Pro Glu | 2496 |

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| | 820 | 825 | 830 | |
|----|---|-----|-----|------|
| 5 | CCA CTT CCA GTT GAT AAC ATG CCA ACG TTC ACA GTA TTG ACT CCT CAC Pro Leu Pro Val Asp Asn Met Pro Thr Phe Thr Val Leu Thr Pro His 835 840 845 | | | 2544 |
| | TAC GCG GAA AGA ATT CTG CTG TCA TTA AGA GAA ATT ATT CGT GAA GAT Tyr Ala Glu Arg Ile Leu Leu Ser Leu Arg Glu Ile Ile Arg Glu Asp 850 855 860 | | | 2592 |
| 10 | GAC CAA TTT TCT AGA GTT ACT CTT TTA GAA TAT CTA AAA CAA TTA CAT Asp Gln Phe Ser Arg Val Thr Leu Leu Glu Tyr Leu Lys Gln Leu His 865 870 875 880 | | | 2640 |
| | CCC GTT GAA TGG GAA TGT TTT GTT AAG GAT ACT AAG ATT TTG GCT GAA Pro Val Glu Trp Glu Cys Phe Val Lys Asp Thr Lys Ile Leu Ala Glu 885 890 895 | | | 2688 |
| 15 | GAA ACC GCT GCC TAT GAA GGA AAT GAA AAT GAA GCT GAA AAG GAA GAT Glu Thr Ala Ala Tyr Glu Gly Asn Glu Asn Glu Ala Glu Lys Glu Asp 900 905 910 | | | 2736 |
| 20 | GCT TTG AAA TCT CAA ATC GAT GAT TTG CCA TTT TAT TGT ATT GGT TTT Ala Leu Lys Ser Gln Ile Asp Asp Leu Pro Phe Tyr Cys Ile Gly Phe 915 920 925 | | | 2784 |
| | AAA TCT GCT GCT CCA GAA TAT ACA CTT CGT ACG AGA ATT TGG GCT TCT Lys Ser Ala Ala Pro Glu Tyr Thr Leu Arg Thr Arg Ile Trp Ala Ser 930 935 940 | | | 2832 |
| 25 | TTG AGG TCG CAG ACT CTA TAT CGT ACC ATT TCA GGG TTC ATG AAT TAT Leu Arg Ser Gln Thr Leu Tyr Arg Thr Ile Ser Gly Phe Met Asn Tyr 945 950 955 960 | | | 2880 |
| | TCA AGA CCT ATC AAA TTA CTG TAT CGT GTG GAA AAT CCT GAA ATT GTT Ser Arg Ala Ile Lys Leu Leu Tyr Arg Val Glu Asn Pro Glu Ile Val 965 970 975 | | | 2928 |
| 30 | CAA ATG TTT GGT GGT AAT GCT GAA GGC TTA GAA AGA GAG CTA GAA AAG Gln Met Phe Gly Gly Asn Ala Glu Gly Leu Glu Arg Glu Leu Glu Lys 980 985 990 | | | 2976 |
| | ATG GCA AGA AGA AAG TTT AAA TTT TTG GTC TCT ATG CAG AGA TTG GCT Met Ala Arg Arg Lys Phe Lys Phe Leu Val Ser Met Gln Arg Leu Ala 995 1000 1005 | | | 3024 |
| 35 | AAA TTC AAA CCA CAT GAA CTG GAA AAT GCT GAG TTT TTG TTG AGA GCT Lys Phe Lys Pro His Glu Leu Glu Asn Ala Glu Phe Leu Leu Arg Ala 1010 1015 1020 | | | 3072 |
| 40 | TAC CCA GAC TTA CAA ATT GCC TAC TTG GAT GAA GAG CCA CCT TTG ACT Tyr Pro Asp Leu Gln Ile Ala Tyr Leu Asp Glu Glu Pro Pro Leu Thr 1025 1030 1035 1040 | | | 3120 |
| | GAA GGT GAG GAG CCA AGA ATC TAT TCC GCT TTG ATT GAT GGA CAT TGT Glu Gly Glu Glu Pro Arg Ile Tyr Ser Ala Leu Ile Asp Gly His Cys 1045 1050 1055 | | | 3168 |
| 45 | GAA ATT CTA GAT AAT GGT CGT AGA CGT CCC AAG TTT AGA GTT CAA TTA Glu Ile Leu Asp Asn Gly Arg Arg Pro Lys Phe Arg Val Gln Leu 1060 1065 1070 | | | 3216 |
| 50 | TCT GGT AAC CCA ATT CTT GGT GAC GGT AAA TCT GAT AAC CAA AAC CAT Ser Gly Asn Pro Ile Leu Gly Asp Gly Lys Ser Asp Asn Gln Asn His 1075 1080 1085 | | | 3244 |

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| | GCT TTG ATT TTT TAC AGA GGT GAA TAC ATT CAA TTA ATT GAT GCC AAC Ala Leu Ile Phe Tyr Arg Gly Glu Tyr Ile Gin Leu Ile Asp Ala Asn 1090 1095 1100 | 3312 |
| 5 | CAA GAT AAC TAC TTG GAA GAA TGT CTG AAG ATT AGA TCT GTA TTG GCT Gln Asp Asn Tyr Leu Glu Glu Cys Leu Lys Ile Arg Ser Val Leu Ala 1105 1110 1115 1120 | 3360 |
| 10 | GAA TTT GAG GAA TTG AAC GTT GAA CAA GTT AAT CCA TAT GCT CCC GGT Glu Phe Glu Glu Leu Asn Val Glu Gln Val Asn Pro Tyr Ala Pro Gly 1125 1130 1135 | 3408 |
| | TTA AGC TAT GAG GAG CAA ACA ACT AAT CAT CCT GTT GCT ATT GTT GGT Leu Arg Tyr Glu Glu Gln Thr Thr Asn His Pro Val Ala Ile Val Gly 1140 1145 1150 | 3456 |
| 15 | GCC AGA GAA TAC ATT TTC TCT GAA AAC TCT GGT GTG CTG GGT GAT GTG Ala Arg Glu Tyr Ile Phe Ser Glu Asn Ser Gly Val Leu Gly Asp Val 1155 1160 1165 | 3504 |
| | GCC GCT GGT AAA GAA CAA ACT TTT GGT ACA TTA TTT GCG CGT ACT TTA Ala Ala Gly Lys Glu Gln Thr Phe Gly Thr Leu Phe Ala Arg Thr Leu 1170 1175 1180 | 3552 |
| 20 | TCT CAA ATT GGT GGT AAA TTG CAT TAT GGT CAT CCG GAT TTC ATT AAT Ser Gln Ile Gly Gly Lys Leu His Tyr Gly His Pro Asp Phe Ile Asn 1185 1190 1195 1200 | 3600 |
| 25 | GCT ACG TTT ATG ACC ACT AGA GGT GGT GTT TCC AAA GCA CAA AAG GGT Ala Thr Phe Met Thr Thr Arg Gly Gly Val Ser Lys Ala Gln Lys Gly 1205 1210 1215 | 3648 |
| | TTG CAT TTA AAC GAA GAT ATT TAT GCT GGT ATG AAT GCT ATG CTT CGT Leu His Leu Asn Glu Asp Ile Tyr Ala Gly Met Asn Ala Met Leu Arg 1220 1225 1230 | 3696 |
| 30 | GGT GGT CGT ATC AAG CAT TGT GAG TAT TAT CAA TGT GGT AAA GGT AGA Gly Gly Arg Ile Lys His Cys Glu Tyr Tyr Gln Cys Gly Lys Gly Arg 1235 1240 1245 | 3744 |
| | GAT TTG GGT TTC GGT ACA ATT CTA AAT TTC ACT ACT AAG ATT GGT GCT Asp Leu Gly Phe Gly Thr Ile Leu Asn Phe Thr Thr Lys Ile Gly Ala 1250 1255 1260 | 3792 |
| 35 | GGT ATG GGT GAA CAA ATG TTA TCT CGT GAA TAT TAT TAT CTG GGT ACC Gly Met Gly Glu Gln Met Leu Ser Arg Glu Tyr Tyr Leu Gly Thr 1265 1270 1275 1280 | 3840 |
| 40 | CAA TTA CCA GTG GAC CGT TTC CTA ACA TTC TAT TAT GCC CAT CCT GGT Gln Leu Pro Val Asp Arg Phe Leu Thr Phe Tyr Tyr Ala His Pro Gly 1285 1290 1295 | 3888 |
| | TTC CAT TTG AAC AAC TTG TTC ATT CAA TTA TCT TTG CAA ATG TTT ATG Phe His Leu Asn Asn Leu Phe Ile Gln Leu Ser Leu Gln Met Phe Met 1300 1305 1310 | 3936 |
| 45 | TTG ACT TTG GTG AAT TTA TCT TCC TTG GCC CAT GAA TCT ATT ATG TGT Leu Thr Leu Val Asn Leu Ser Ser Leu Ala His Glu Ser Ile Met Cys 1315 1320 1325 | 3984 |
| | ATT TAC GAT AGG AAC AAA CCA AAA ACA GAT GTT TTG GTT CCA ATT GGG Ile Tyr Asp Arg Asn Lys Pro Lys Thr Asp Val Leu Val Pro Ile Gly 1330 1335 1340 | 4032 |
| 50 | | |

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|----|---|------|
| | TGT TAC AAC TTC CAA CCT GCG GTT GAT TGG GTG AGA CGT TAT ACA TTG Cys Tyr Asn Phe Gln Pro Ala Val Asp Trp Val Arg Arg Tyr Thr Leu 1345 1350 1355 1360 | 4080 |
| 5 | TCT ATT TTC ATT GTT TTC TGG ATT GCC TTC GTT CCT ATT GTT GTT CAA Ser Ile Phe Ile Val Phe Trp Ile Ala Phe Val Pro Ile Val Val Gln 1365 1370 1375 | 4128 |
| 10 | GAA CTA ATT GAA CGT GGT CTA TGG AAA GCC ACC CAA AGA TTT TTC TGC Glu Leu Ile Glu Arg Gly Leu Trp Lys Ala Thr Gln Arg Phe Phe Cys 1380 1385 1390 | 4176 |
| | CAC CTA TTA TCA TTA TCC CCT ATG TTC GAA GTG TTT GCG GGC CAA ATC His Leu Leu Ser Leu Ser Pro Met Phe Glu Val Phe Ala Gly Gln Ile 1395 1400 1405 | 4224 |
| 15 | TAC TCT TCT GCG TTA TTA AGT GAT TTA GCA ATT GGT GGT GCT CGT TAT Tyr Ser Ser Ala Leu Leu Ser Asp Leu Ala Ile Gly Gly Ala Arg Tyr 1410 1415 1420 | 4272 |
| | ATA TCC ACC GGT CGT GGT TTT GCA ACT TCT CGT ATA CCA TTT TCA ATT Ile Ser Thr Gly Arg Gly Phe Ala Thr Ser Arg Ile Pro Phe Ser Ile 1425 1430 1435 1440 | 4320 |
| 20 | TTG TAT TCA AGA TTT GCA GGA TCT GCT ATC TAC ATG GGT GCA AGA TCA Leu Tyr Ser Arg Phe Ala Gly Ser Ala Ile Tyr Met Gly Ala Arg Ser 1445 1450 1455 | 4368 |
| 25 | ATG TTA ATG TTG CTG TTC GGT ACT GTC GCA CAT TGG CAA GCT CCA CTA Met Leu Met Leu Phe Gly Thr Val Ala His Trp Gln Ala Pro Leu 1460 1465 1470 | 4416 |
| | CTG TGG TTT TGG GCC TCT CTA TCT TCA TTA ATT TTT GCG CCT TTC GTT Leu Trp Phe Trp Ala Ser Leu Ser Ser Leu Ile Phe Ala Pro Phe Val 1475 1480 1485 | 4464 |
| 30 | TTC AAT CCA CAT CAG TTT GCT TGG GAA GAT TTC TTT TTG GAT TAC AGG Phe Asn Pro His Gln Phe Ala Trp Glu Asp Phe Phe Leu Asp Tyr Arg 1490 1495 1500 | 4512 |
| | GAT TAT ATC AGA TGG TTA TCA AGA GGT AAT AAT CAA TAT CAT AGA AAC Asp Tyr Ile Arg Trp Leu Ser Arg Gly Asn Asn Gln Tyr His Arg Asn 1505 1510 1515 1520 | 4560 |
| 35 | TCG TGG ATT GGT TAC GTG AGG ATG TCT AGG GCA CGT ATT ACT GGG TTT Ser Trp Ile Gly Tyr Val Arg Met Ser Arg Ala Arg Ile Thr Gly Phe 1525 1530 1535 | 4608 |
| 40 | AAA CGT AAA CTG GTT GGC GAT GAA TCT GAG AAA GCT GCT GGT GAC GCA Lys Arg Lys Leu Val Gly Asp Glu Ser Glu Lys Ala Ala Gly Asp Ala 1540 1545 1550 | 4656 |
| | AGC AGG CCT CAT AGA ACC AAT TTG ATC ATG GCT GAA ATC ATA CCC TGT Ser Arg Ala His Arg Thr Asn Leu Ile Met Ala Glu Ile Ile Pro Cys 1555 1560 1565 | 4704 |
| 45 | GCA ATT TAT GCA GCT GGT TGT TTT ATT GCC TTC ACG TTT ATT AAT GCT Ala Ile Tyr Ala Ala Gly Cys Phe Ile Ala Phe Thr Phe Ile Asn Ala 1570 1575 1580 | 4752 |
| | CAA ACC GGT GTC AAG ACT ACT GAT GAT AGG GTG AAT TCT GTT TTA Gln Thr Gly Val Lys Thr Thr Asp Asp Asp Arg Val Asn Ser Val Leu 1585 1590 1595 1600 | 4800 |
| 50 | CGT ATC ATC ATT TGT ACC TTG GCG CCA ATC GCC GTT AAC CTC GGT GTT | 4848 |

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|----|---|------|
| | Arg Ile Ile Ile Cys Thr Leu Ala Pro Ile Ala Val Asn Leu Gly Val 1605 1610 1615 | |
| 5 | CTA TTC TTC TGT ATG GGT ATG TCA TGC TGC TCT GGT CCC TTA TTT GGT Leu Phe Cys Met Gly Met Ser Cys Cys Ser Gly Pro Leu Phe Gly 1620 1625 1630 | 4896 |
| | ATG TGT TGT AAG AAG ACA GGT TCT GTA ATG GCT GGA ATT GCC CAC GGT Met Cys Cys Lys Thr Gly Ser Val Met Ala Gly Ile Ala His Gly 1635 1640 1645 | 4944 |
| 10 | CTT GCT GTT ATT GTC CAC ATT GCC TTT TTC ATT GTC ATG TGG GTT TTG Val Ala Val Ile Ala Phe Ile Val Met Trp Val Leu 1650 1655 1660 | 4992 |
| | GAG AGC TTC AAC TTT GTT AGA ATG TTA ATC GGA GTC GTT ACT TGT ATC Glu Ser Phe Asn Phe Val Arg Met Leu Ile Gly Val Val Thr Cys Ile 1665 1670 1675 1680 | 5040 |
| 15 | CAA TGT CAA AGA CTC ATT TTT CAT TGC ATG ACA GCG TTA ATG TTG ACT Gln Cys Gln Arg Leu Ile Phe His Cys Met Thr Ala Leu Met Leu Thr 1685 1690 1695 | 5088 |
| 20 | CGT GAA TTT AAA AAC GAT CAT GCC AAT ACA GCC TTC TGG ACT GGT AAG Arg Glu Phe Lys Asn Asp His Ala Asn Thr Ala Phe Trp Thr Gly Lys 1700 1705 1710 | 5136 |
| | TGG TAT GGT AAA GGT ATG GGT TAC ATG GCT TGG ACC CAG CCA AGT AGA Trp Tyr Gly Lys Gly Met Gly Tyr Met Ala Trp Thr Gln Pro Ser Arg 1715 1720 1725 | 5184 |
| 25 | GAA TTA ACC GCC AAG GTA ATT GAG CTT TCA GAA TTT GCA GCT GAT TTT Glu Leu Thr Ala Lys Val Ile Glu Leu Ser Glu Phe Ala Ala Asp Phe 1730 1735 1740 | 5232 |
| 30 | GTT CTA CGT CAT GTG ATT TTA ATC TGT CAA CTG CCA CTC ATT ATA ATC Val Leu Gly His Val Ile Leu Ile Cys Gln Leu Pro Leu Ile Ile Ile 1745 1750 1755 1760 | 5280 |
| | CCA AAA ATA GAT AAA TTC CAC TCG ATT ATG CTA TTC TGG CTA AAG CCC Pro Lys Ile Asp Lys Phe His Ser Ile Met Leu Phe Trp Leu Lys Pro 1765 1770 1775 | 5328 |
| 35 | TCT CGT CAA ATT CGT CCC CCA ATT TAC TCT CTG AAG CAA ACT CGT TTG Ser Arg Gln Ile Arg Pro Pro Ile Tyr Ser Leu Lys Gln Thr Arg Leu 1780 1785 1790 | 5376 |
| | CGT AAG CGT ATG GTC AAG AAG TAC TGC TCT TTG TAC TTT TTA GTA TTG Arg Lys Arg Met Val Lys Lys Tyr Cys Ser Leu Tyr Phe Leu Val Leu 1795 1800 1805 | 5424 |
| 40 | GCT ATT TTT GCA GGA TGC ATT ATT GGT CCT GCT GTA GCC TCT GCT AAG Ala Ile Phe Ala Gly Cys Ile Ile Gly Pro Ala Val Ala Ser Ala Lys 1810 1815 1820 | 5472 |
| | ATC CAC AAA CAC ATT GGA GAT TCA TTG GAT GGC GTT GTT CAC AAT CTA Ile His Lys His Ile Gly Asp Ser Leu Asp Gly Val Val His Asn Leu 1825 1830 1835 1840 | 5520 |
| 45 | TTC CAA CCA ATA AAT ACA ACC AAT AAT GAC ACT GGT TCC CAA ATG TCA Phe Gln Pro Ile Asn Thr Thr Asn Asn Asp Thr Gly Ser Gln Met Ser 1845 1850 1855 | 5568 |
| 50 | ACT TAT CAA AGT CAC TAC TAT ACT CAT ACG CCA TCA TTA AAG ACC TGG Thr Tyr Gln Ser His Tyr Tyr His Thr Pro Ser Leu Lys Thr Trp | 5616 |

1860

1865

1870

5631

5 TCA ACT ATA AAA TAA
 Ser Thr Ile Lys
 1875

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1876 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

15 Met Asn Thr Asp Gln Gln Pro Tyr Gln Gly Gln Thr Asp Tyr Thr Gln
 1 5 10 15

Gly Pro Gly Asn Gly Gln Ser Gln Glu Gln Asp Tyr Asp Gln Tyr Gly
 20 25 30

20 Gln Pro Leu Tyr Pro Ser Gln Ala Asp Gly Tyr Tyr Asp Pro Asn Val
 35 40 45

Ala Ala Gly Thr Glu Ala Asp Met Tyr Gly Gln Gln Pro Pro Asn Glu
 50 55 60

25 Ser Tyr Asp Gln Asp Tyr Thr Asn Gly Glu Tyr Tyr Gly Gln Pro Pro
 65 70 75 80

Asn Met Ala Ala Gln Asp Gly Glu Asn Phe Ser Asp Phe Ser Ser Tyr
 85 90 95

30 Gly Pro Pro Gly Thr Pro Gly Tyr Asp Ser Tyr Gly Gly Gln Tyr Thr
 100 105 110

Ala Ser Gln Met Ser Tyr Gly Glu Pro Asn Ser Ser Gly Thr Ser Thr
 115 120 125

35 Pro Ile Tyr Gly Asn Tyr Asp Pro Asn Ala Ile Ala Met Ala Leu Pro
 130 135 140

Asn Glu Pro Tyr Pro Ala Trp Thr Ala Asp Ser Gln Ser Pro Val Ser
 145 150 155 160

Ile Glu Gln Ile Glu Asp Ile Phe Ile Asp Leu Thr Asn Arg Leu Gly
 165 170 175

40 Phe Gln Arg Asp Ser Met Arg Asn Met Phe Asp His Phe Met Val Leu
 180 185 190

Leu Asp Ser Arg Ser Ser Arg Met Ser Pro Asp Gln Ala Leu Leu Ser
 195 200 205

45 Leu His Ala Asp Tyr Ile Gly Gly Asp Thr Ala Asn Tyr Lys Lys Trp
 210 215 220

Tyr Phe Ala Ala Gln Leu Asp Met Asp Asp Glu Ile Gly Phe Arg Asn
 225 230 235 240

50 Met Ser Leu Gly Lys Leu Ser Arg Lys Ala Arg Lys Ala Lys Lys Lys
 245 250 255

Asn Lys Lys Ala Met Glu Glu Ala Asn Pro Glu Asp Thr Glu Glu Thr
 260 265 270
 5 Leu Asn Lys Ile Glu Gly Asp Asn Ser Leu Glu Ala Ala Asp Phe Arg
 275 280 285
 Trp Lys Ala Lys Met Asn Gln Leu Ser Pro Leu Glu Arg Val Arg His
 290 295 300
 10 Ile Ala Leu Tyr Leu Leu Cys Trp Gly Glu Ala Asn Gln Val Arg Phe
 305 310 315 320
 Thr Ala Glu Cys Leu Cys Phe Ile Tyr Lys Cys Ala Leu Asp Tyr Leu
 325 330 335
 15 Asp Ser Pro Leu Cys Gln Gln Arg Gln Glu Pro Met Pro Glu Gly Asp
 340 345 350
 Phe Leu Asn Arg Val Ile Thr Pro Ile Tyr His Phe Ile Arg Asn Gln
 355 360 365
 20 Val Tyr Glu Ile Val Asp Gly Arg Phe Val Lys Arg Glu Arg Asp His
 370 375 380
 Asn Lys Ile Val Gly Tyr Asp Asp Leu Asn Gln Leu Phe Trp Tyr Pro
 385 390 395 400
 25 Glu Gly Ile Ala Lys Ile Val Leu Glu Asp Gly Thr Lys Leu Ile Glu
 405 410 415
 Leu Pro Leu Glu Glu Arg Tyr Leu Arg Leu Gly Asp Val Val Trp Asp
 420 425 430
 30 Asp Val Phe Phe Lys Thr Tyr Lys Glu Thr Arg Thr Trp Leu His Leu
 435 440 445
 Val Thr Asn Phe Asn Arg Ile Trp Val Met His Ile Ser Ile Phe Trp
 450 455 460
 35 Met Tyr Phe Ala Tyr Asn Ser Pro Thr Phe Tyr Thr His Asn Tyr Gln
 465 470 475 480
 Gln Leu Val Asp Asn Gln Pro Leu Ala Ala Tyr Lys Trp Ala Ser Cys
 485 490 495
 40 Ala Leu Gly Gly Thr Val Ala Ser Leu Ile Gln Ile Val Ala Thr Leu
 500 505 510
 Cys Glu Trp Ser Phe Val Pro Arg Lys Trp Ala Gly Ala Gln His Leu
 515 520 525
 45 Ser Arg Arg Phe Trp Phe Leu Cys Ile Ile Phe Gly Ile Asn Leu Gly
 530 535 540
 Pro Ile Ile Phe Val Phe Ala Tyr Asp Lys Asp Thr Val Tyr Ser Thr
 545 550 555 560
 50 Ala Ala His Val Val Ala Ala Val Met Phe Phe Val Ala Val Ala Thr
 565 570 575
 Ile Ile Phe Phe Ser Ile Met Pro Leu Gly Gly Leu Phe Thr Ser Tyr
 580 585 590
 55 Met Lys Lys Ser Thr Arg Arg Tyr Val Ala Ser Gln Thr Phe Thr Ala

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| | 595 | 600 | 605 |
|----|---|-----|-----|
| | Ala Phe Ala Pro Leu His Gly Leu Asp Arg Trp Met Ser Tyr Leu Val | | |
| 5 | 610 615 620 | | |
| | Trp Val Thr Val Phe Ala Ala Lys Tyr Ser Glu Ser Tyr Tyr Phe Leu | | |
| | 625 630 635 640 | | |
| | Val Leu Ser Leu Arg Asp Pro Ile Arg Ile Leu Ser Thr Thr Ala Met | | |
| 10 | 645 650 655 | | |
| | Arg Cys Thr Gly Glu Tyr Trp Trp Gly Ala Val Leu Cys Lys Val Gln | | |
| | 660 665 670 | | |
| | Pro Lys Ile Val Leu Gly Leu Val Ile Ala Thr Asp Phe Ile Leu Phe | | |
| 15 | 675 680 685 | | |
| | Phe Leu Asp Thr Tyr Leu Trp Tyr Ile Ile Val Asn Thr Ile Phe Ser | | |
| | 690 695 700 | | |
| | Val Gly Lys Ser Phe Tyr Leu Gly Ile Ser Ile Leu Thr Pro Trp Arg | | |
| 20 | 705 710 715 720 | | |
| | Asn Ile Phe Thr Arg Leu Pro Lys Arg Ile Tyr Ser Lys Ile Leu Ala | | |
| | 725 730 735 | | |
| | Thr Thr Asp Met Glu Ile Lys Tyr Lys Pro Lys Val Leu Ile Ser Gln | | |
| 25 | 740 745 750 | | |
| | Val Trp Asn Ala Ile Ile Ser Met Tyr Arg Glu His Leu Leu Ala | | |
| | 755 760 765 | | |
| | Ile Asp His Val Gln Lys Leu Leu Tyr His Gln Val Pro Ser Glu Ile | | |
| 30 | 770 775 780 | | |
| | Glu Gly Lys Arg Thr Leu Arg Ala Pro Thr Phe Phe Val Ser Gln Asp | | |
| | 785 790 795 800 | | |
| | Asp Asn Asn Phe Glu Thr Glu Phe Phe Pro Arg Asp Ser Glu Ala Glu | | |
| 35 | 805 810 815 | | |
| | Arg Arg Ile Ser Phe Phe Ala Gln Ser Leu Ser Thr Pro Ile Pro Glu | | |
| | 820 825 830 | | |
| | Pro Leu Pro Val Asn Met Pro Thr Phe Thr Val Leu Thr Pro His | | |
| 40 | 835 840 845 | | |
| | Tyr Ala Glu Arg Ile Leu Leu Ser Leu Arg Glu Ile Ile Arg Glu Asp | | |
| | 850 855 860 | | |
| | Asp Gln Phe Ser Arg Val Thr Leu Leu Glu Tyr Leu Lys Gln Leu His | | |
| 45 | 865 870 875 880 | | |
| | Pro Val Glu Trp Glu Cys Phe Val Lys Asp Thr Lys Ile Leu Ala Glu | | |
| | 885 890 895 | | |
| | Glu Thr Ala Ala Tyr Glu Gly Asn Glu Asn Glu Ala Glu Lys Glu Asp | | |
| 50 | 900 905 910 | | |
| | Ala Leu Lys Ser Gln Ile Asp Asp Leu Pro Phe Tyr Cys Ile Gly Phe | | |
| | 915 920 925 | | |
| | Lys Ser Ala Ala Pro Glu Tyr Thr Leu Arg Thr Arg Ile Trp Ala Ser | | |
| 55 | 930 935 940 | | |

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Leu Arg Ser Gln Thr Leu Tyr Arg Thr Ile Ser Gly Phe Met Asn Tyr
 945 950 955 960
 5 Ser Arg Ala Ile Lys Leu Leu Tyr Arg Val Glu Asn Pro Glu Ile Val
 965 970 975
 Gin Met Phe Gly Gly Asn Ala Glu Gly Leu Glu Arg Glu Leu Glu Lys
 980 985 990
 10 Met Ala Arg Arg Lys Phe Lys Phe Leu Val Ser Met Gln Arg Leu Ala
 995 1000 1005
 Lys Phe Lys Pro His Glu Leu Glu Asn Ala Glu Phe Leu Leu Arg Ala
 1010 1015 1020
 15 Tyr Pro Asp Leu Gln Ile Ala Tyr Leu Asp Glu Glu Pro Pro Leu Thr
 1025 1030 1035 1040
 Glu Gly Glu Glu Pro Arg Ile Tyr Ser Ala Leu Ile Asp Gly His Cys
 1045 1050 1055
 20 Glu Ile Leu Asp Asn Gly Arg Arg Pro Lys Phe Arg Val Gln Leu
 1060 1065 1070
 Ser Gly Asn Pro Ile Leu Gly Asp Gly Lys Ser Asp Asn Gln Asn His
 1075 1080 1085
 25 Ala Leu Ile Phe Tyr Arg Gly Glu Tyr Ile Gln Leu Ile Asp Ala Asn
 1090 1095 1100
 Gln Asp Asn Tyr Leu Glu Glu Cys Leu Lys Ile Arg Ser Val Leu Ala
 1105 1110 1115 1120
 30 Glu Phe Glu Glu Leu Asn Val Glu Gln Val Asn Pro Tyr Ala Pro Gly
 1125 1130 1135
 Leu Arg Tyr Glu Glu Gln Thr Thr Asn His Pro Val Ala Ile Val Gly
 1140 1145 1150
 35 Ala Arg Glu Tyr Ile Phe Ser Glu Asn Ser Gly Val Leu Gly Asp Val
 1155 1160 1165
 Ala Ala Gly Lys Glu Gln Thr Phe Gly Thr Leu Phe Ala Arg Thr Leu
 1170 1175 1180
 40 Ser Gln Ile Gly Gly Lys Leu His Tyr Gly His Pro Asp Phe Ile Asn
 1185 1190 1195 1200
 Ala Thr Phe Met Thr Thr Arg Gly Val Ser Lys Ala Gln Lys Gly
 1205 1210 1215
 Leu His Leu Asn Glu Asp Ile Tyr Ala Gly Met Asn Ala Met Leu Arg
 45 1220 1225 1230
 Gly Gly Arg Ile Lys His Cys Glu Tyr Tyr Gln Cys Gly Lys Gly Arg
 1235 1240 1245
 50 Asp Leu Gly Phe Gly Thr Ile Leu Asn Phe Thr Thr Lys Ile Gly Ala
 1250 1255 1260
 Gly Met Gly Glu Gln Met Leu Ser Arg Glu Tyr Tyr Tyr Leu Gly Thr
 1265 1270 1275 1280
 55 Gln Leu Pro Val Asp Arg Phe Leu Thr Phe Tyr Tyr Ala His Pro Gly
 1285 1290 1295

Phe His Leu Asn Asn Leu Phe Ile Gln Leu Ser Leu Gln Met Phe Met
 1300 1305 1310
 5 Leu Thr Leu Val Asn Leu Ser Ser Leu Ala His Glu Ser Ile Met Cys
 1315 1320 1325
 Ile Tyr Asp Arg Asn Lys Pro Lys Thr Asp Val Leu Val Pro Ile Gly
 1330 1335 1340
 10 Cys Tyr Asn Phe Gln Pro Ala Val Asp Trp Val Arg Arg Tyr Thr Leu
 1345 1350 1355 1360
 Ser Ile Phe Ile Val Phe Trp Ile Ala Phe Val Pro Ile Val Val Gln
 1365 1370 1375
 15 Glu Leu Ile Glu Arg Gly Leu Trp Lys Ala Thr Gln Arg Phe Phe Cys
 1380 1385 1390
 His Leu Leu Ser Leu Ser Pro Met Phe Glu Val Phe Ala Gly Gln Ile
 1395 1400 1405
 20 Tyr Ser Ser Ala Leu Leu Ser Asp Leu Ala Ile Gly Gly Ala Arg Tyr
 1410 1415 1420
 Ile Ser Thr Gly Arg Gly Phe Ala Thr Ser Arg Ile Pro Phe Ser Ile
 1425 1430 1435 1440
 25 Leu Tyr Ser Arg Phe Ala Gly Ser Ala Ile Tyr Met Gly Ala Arg Ser
 1445 1450 1455
 Met Leu Met Leu Leu Phe Gly Thr Val Ala His Trp Gln Ala Pro Leu
 1460 1465 1470
 30 Leu Trp Phe Trp Ala Ser Leu Ser Ser Leu Ile Phe Ala Pro Phe Val
 1475 1480 1485
 Phe Asn Pro His Gln Phe Ala Trp Glu Asp Phe Phe Leu Asp Tyr Arg
 1490 1495 1500
 35 Asp Tyr Ile Arg Trp Leu Ser Arg Gly Asn Asn Gln Tyr His Arg Asn
 1505 1510 1515 1520
 Ser Trp Ile Gly Tyr Val Arg Met Ser Arg Ala Arg Ile Thr Gly Phe
 1525 1530 1535
 40 Lys Arg Lys Leu Val Gly Asp Glu Ser Glu Lys Ala Ala Gly Asp Ala
 1540 1545 1550
 Ser Arg Ala His Arg Thr Asn Leu Ile Met Ala Glu Ile Ile Pro Cys
 1555 1560 1565
 45 Ala Ile Tyr Ala Ala Gly Cys Phe Ile Ala Phe Thr Phe Ile Asn Ala
 1570 1575 1580
 Gln Thr Gly Val Lys Thr Thr Asp Asp Asp Arg Val Asn Ser Val Leu
 1585 1590 1595 1600
 50 Arg Ile Ile Ile Cys Thr Leu Ala Pro Ile Ala Val Asn Leu Gly Val
 1605 1610 1615
 Leu Phe Phe Cys Met Gly Met Ser Cys Cys Ser Gly Pro Leu Phe Gly
 1620 1625 1630
 55 Met Cys Cys Lys Lys Thr Gly Ser Val Met Ala Gly Ile Ala His Gly

| | 1635 | 1640 | 1645 |
|----|---|------|------|
| 5 | Val Ala Val Ile Val His Ile Ala Phe Phe Ile Val Met Trp Val Leu 1650 | 1655 | 1660 |
| | Glu Ser Phe Asn Phe Val Arg Met Leu Ile Gly Val Val Thr Cys Ile 1665 | 1670 | 1675 |
| 10 | Gln Cys Gln Arg Leu Ile Phe His Cys Met Thr Ala Leu Met Leu Thr 1685 | 1690 | 1695 |
| | Arg Glu Phe Lys Asn Asp His Ala Asn Thr Ala Phe Trp Thr Gly Lys 1700 | 1705 | 1710 |
| 15 | Trp Tyr Gly Met Gly Tyr Met Ala Trp Thr Gln Pro Ser Arg 1715 | 1720 | 1725 |
| | Glu Leu Thr Ala Lys Val Ile Glu Leu Ser Glu Phe Ala Ala Asp Phe 1730 | 1735 | 1740 |
| 20 | Val Leu Gly His Val Ile Leu Ile Cys Gln Leu Pro Leu Ile Ile Ile 1745 | 1750 | 1755 |
| | Pro Lys Ile Asp Lys Phe His Ser Ile Met Leu Phe Trp Leu Lys Pro 1765 | 1770 | 1775 |
| 25 | Ser Arg Gln Ile Arg Pro Pro Ile Tyr Ser Leu Lys Gln Thr Arg Leu 1780 | 1785 | 1790 |
| | Arg Lys Arg Met Val Lys Lys Tyr Cys Ser Leu Tyr Phe Leu Val Leu 1795 | 1800 | 1805 |
| 30 | Ala Ile Phe Ala Gly Cys Ile Ile Gly Pro Ala Val Ala Ser Ala Lys 1810 | 1815 | 1820 |
| | Ile His Lys His Ile Gly Asp Ser Leu Asp Gly Val Val His Asn Leu 1825 | 1830 | 1835 |
| 35 | Phe Gln Pro Ile Asn Thr Thr Asn Asn Asp Thr Gly Ser Gln Met Ser 1845 | 1850 | 1855 |
| | Thr Tyr Gln Ser His Tyr Tyr Thr His Thr Pro Ser Leu Lys Thr Trp 1860 | 1865 | 1870 |
| 40 | Ser Thr Ile Lys 1875 | | |
| 45 | | | |

Claims

1. A substantially pure ECB binding peptide comprising at least 46 contiguous amino acid residues of SEQ ID NO:2.
2. A substantially pure ECB binding peptide, as in Claim 1 comprising the amino acid sequence defined by residues 605 to 650 of SEQ ID NO:2.
3. An isolated nucleic acid compound encoding a peptide of Claim 1 or Claim 2.
4. An isolated nucleic acid encoding a peptide of Claim 1 wherein said nucleic acid has a sequence selected from the group consisting of:

(a) (a) residues 1747 to 2016 of SEQ ID NO:1; or
(b) a nucleic acid compound complementary to (a).

5. A vector comprising an isolated nucleic acid compound of Claim 3.
6. A host cell containing a vector of Claim 5.
7. A method for constructing a recombinant host cell having the potential to express an ECB binding domain of SEQ ID NO:2, said method comprising introducing into said host cell by any suitable means a vector of Claim 5.
- 10 8. A method for expressing an ECB binding domain of SEQ ID NO:2 in the recombinant host cell of Claim 7, said method comprising culturing said recombinant host cell under conditions suitable for gene expression.
- 15 9. A method for identifying compounds that bind an ECB binding domain, comprising the steps of:
 - a) admixing in a suitable reaction buffer
 - i) a substantially pure ECB binding peptide, as claimed in Claim 1; and
 - ii) a test inhibitory compound;
 - 20 b) measuring by any suitable means a binding between said peptide and said compound.

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Europäisches Patentamt
European Patent Office
Office europeen des brevets



(11) EP 0 931 834 A3

(12)

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C12N 1/15, C12N 1/21,
C12Q 1/48

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(54) **Echinocandin binding domain of 1,3-Beta-glucan synthase**

(57) The invention relates to a substantially purified ECB binding domain of 1,3- β -glucan synthase, comprising an at least 46 amino acid peptide fragment or fusion protein of glucan synthase that binds echinocandins,

useful in a method for identifying new antifungal compounds. Also disclosed are nucleic acid molecules that encode said peptide.

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European Patent
Office

EUROPEAN SEARCH REPORT

Application Number
EP 98 31 0497

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|---|--|-------------------|---|
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| Y | DOUGLAS C M ET AL : "Identification of the FKS1 gene of Candida albicans as the essential target of 1,3-beta-D-glucan synthase inhibitors" ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, vol. 41, no. 11, November 1997 (1997-11), pages 2471-2479, XP000107858 United States * abstract * | 9 | |
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| C12N C12Q | | | |
| The present search report has been drawn up for all claims | | | |
| Place of search | Date of completion of the search | Examiner | |
| MUNICH | 2 May 2002 | Meacock, S | |
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| X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document | | | |
| T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons S : member of the same patent family, corresponding document | | | |

**ANNEX TO THE EUROPEAN SEARCH REPORT
ON EUROPEAN PATENT APPLICATION NO.**

EP 98 31 0497

This annex lists the patent family members relating to the patent documents cited in the above-mentioned European search report. The members are as contained in the European Patent Office EDP file on The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

02-05-2002

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| | | EP | 0724644 A1 | 07-08-1996 |
| | | JP | 9504431 T | 06-05-1997 |
| | | WO | 9510625 A1 | 20-04-1995 |
| | | US | 5821353 A | 13-10-1998 |

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For more details about this annex : see Official Journal of the European Patent Office, No. 12/82